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INVENTOR(S)					
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)			
Laurie Martha	SCANLIN STONE	Arvada, CO Fort Collins, CO			
<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
QUINOA PROTEIN CONCENTRATE, PRODUCTION AND FUNCTIONALITY					
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OR					
<input checked="" type="checkbox"/> Firm or Individual Name		GREENLEE, WINNER AND SULLIVAN, P.C.			
Address		5370 Manhattan Circle, Ste. 201			
Address					
City	Boulder	State	CO	ZIP	80303
Country	US	Telephone	(303) 499-8080	Fax	(303) 499-8089
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Respectfully submitted,

SIGNATURE

Heeja Yoo-Warren

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Inventors:

Laurie Scanlin
Martha Stone

QUINOA PROTEIN CONCENTRATE, PRODUCTION AND FUNCTIONALITY

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N. Kemper

Prepared by:

GREENLEE, WINNER AND SULLIVAN, P.C.
5370 Manhattan Circle, Ste. 201
Boulder, Colorado 80303

(303) 499-8080
FAX: (303) 499-8089

Attachment No. 132-03P

5 QUINOA PROTEIN CONCENTRATE, PRODUCTION AND FUNCTIONALITY

The present invention relates to a quinoa protein concentrate containing at least about 50 wt % protein and a method of processing quinoa (Genus: *Chenopodium*,
10 Species: *quinoa*, Family: Chenopodiaceae) grain (also called quinoa seed, grain-like seed, pseudocereal, and fruit) to produce such protein concentrate, oil, starch, and fiber.

15 BACKGROUND OF THE INVENTION

Plant proteins, processed from cereal grains and legumes, are profitable ingredients in a wide variety of commercial food products, pet foods, and animal feed. Examples of the plant proteins that are currently available are soy protein concentrate,
20 isolated soy protein, wheat gluten, rice, and corn proteins (Food Master 2003).

However, plant proteins are often limiting in one or more essential amino acids. For example, the plant proteins of wheat, rice and corn are limiting in lysine (Hoseney 1986), whereas, soy protein is limiting in methionine and cystine (Haard and Chism
25 1996). Though, well processed isolated soy proteins and soy protein concentrates have been found to be equivalent to animal protein in regards to the needs of human nutrition (Young 1991).

Yet, the following eight foods that are a good source of animal or plant protein
30 account for 90% of all food allergenic reactions: soy, wheat, eggs, milk, peanut, treenut, fish and shellfish (FAAN 2003). Food allergens are a serious concern because

essential nutrients for proper health can be missing with a narrowed food choice, in addition to the life-threatening concern of anaphylactic shock in highly sensitive individuals. Allergens are problematic for food producers because many food ingredients fall into this category and limit product development. The impact that food allergens, including undeclared food allergens, have had on the food industry is remarkable and the FDA has stated that food allergens are a top priority this year (Hefle 2003).

As world food demands steadily increase, production of protein has to be maximized, as well as, augmented. Plant proteins from cereals and legumes represent the main source of proteins and energy supply for both human and animal nutrition. This is partly due to the fact that animal proteins require much higher energy demand for production and are therefore more expensive to produce than plant proteins (Cheftel and others 1985). For example, in order to produce 1 kg of animal protein, 3-20 kg of plant protein is needed. Consequently, as demands for animal protein increase globally, the need for plant protein increases drastically. To meet this need, new protein resources must be developed. Protein-rich crops that give equitable yields in underutilized growing regions are of paramount value for this purpose. Alternatively, new crops can be selected and tested for a protein source.

Since 1975, quinoa has become an alternative crop in North America and Europe for the following reasons (Fleming and Galwey 1995): quinoa has the ability to thrive in marginal soils, where traditional crops cannot, therefore, underutilized growing regions can be cultivated; quinoa has an average protein content of 14.6%, which is higher than traditional cereals, with certain varieties containing protein levels as high as 21.9%; and quinoa has an amino acid composition, protein efficiency ratio, protein digestibility, and nitrogen balance comparable to milk protein, casein. Consequently, it is rare for a plant protein to so closely resemble that of animal origin.

Quinoa protein is particularly high in lysine and methionine, amino acids limiting in cereal grains and legumes, respectively (Koziol 1992). Quinoa protein is also high in

histidine, an essential amino acid for infant development and those with chronic diseases (Ettinger 2000). In South America, it has been used as a weaning food for centuries because of its nutritional attributes and high protein digestibility.

5 Additionally, quinoa is not on the list of recognized food allergens. It is considered free of gluten or prolamins (Fairbanks and others 1990), the protein associated with allergenic reactions in wheat gluten, rye and barley. Prolamins, like gliadins found in wheat, ignite immune responses in patients with gluten-induced enteropathy, also known as celiac disease. Quinoa is a pseudocereal named for its
10 production of small grain-like seeds, although, the actual harvested grain is a single seeded fruit (Shewry 2002). It is a dicotyledonous species not closely related to the monocotyledonous species of true cereal grains like wheat, rye, and barley. As a result of differences in plant taxonomy, quinoa does not contain the harmful amino acid sequences found in wheat, therefore, it is concluded safe for a gluten-free diet
15 (Thompson 2001) and is recommended by the Celiac Disease Foundation and Gluten Intolerance Group. Furthermore, research presented at the International Workshop on Food Supplementation in Food Allergy and Immunity, found that quinoa is immunochemically safe and represents a viable alternative for gluten-free products (Berti and others 2002).

20 Despite the numerous beneficial properties of quinoa as a plant protein source as described above, quinoa grain has not been processed efficiently to extract individual components contained therein. Currently, quinoa is available only as whole grain or ground for a small number of products. Therefore, there is a need in the art to develop
25 a method to process quinoa grains into individual components, i.e., protein, oil, fiber, and starch, which can readily be utilized as nutritional supplements as well as an agent for providing functionality in a variety of food products and animal feeds. The present invention meets this need. The advantage of the invention will be evident in the following description.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a new source of plant protein, termed "quinoa protein concentrate (QPC)", prepared from quinoa (*Chenopodium quinoa* Chenopodiaceae) grain, which contains at least about 50 wt% protein, preferably at least about 70 wt% protein, most preferably at least about 90 wt% protein, on a dry weight basis. The QPC of the invention is high in lysine and histidine, and methionine and cystine, which are often limiting in plant proteins of grains and legumes, respectively. Additionally, quinoa is considered to be non-allergenic, as opposed to key plant allergens, soy and wheat. Therefore, the quinoa protein concentrate is useful as food additives and supplements to provide nutrients as well as necessary functionality in a variety of food products, pet foods and animal feeds. For examples, the QPC can be added in a variety of products such as foods for infants and toddlers, meat analogs, ice creams, whipped toppings, baked products, and salad dressings and the like, to reduce water activity, reduce fat, bind ingredients, emulsify, and/or stabilize foams. The QPC of the invention are particularly useful as an additive to fortify the amino acid composition of corn- or rice-based food products, which are also considered to be non-allergenic, but are either low in protein content or limiting in essential amino acid, lysine. The QPC can be used as a protein source in food products intended for use in subjects who require less- or non-allergenic food products. In addition, QPC can serve as a high quality, plant protein in pet foods and animal feeds like cattle feed, since the FDA banned the use of animal protein in cattle feed, as a preventative measure against bovine spongiform encephalopathy (i.e., BSE or mad cow disease) (James-Preston 2003).

25

Also provided is a process for isolating individual components contained in quinoa (*Chenopodium quinoa* Chenopodiaceae) grain such as protein (termed QPC herein), oil, starch, and fiber. The process comprises the steps of; 1) flaking quinoa grain, 2) extracting the flaked quinoa with a solvent and separating the fraction containing the oil from the remaining defatted quinoa, 3) milling the defatted quinoa, 4) extracting the protein from the milled, defatted quinoa in alkaline solution, and 5)

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separating the fraction containing the protein from the fraction containing starch and fiber, whereby a quinoa protein concentrate containing at least about 50 wt% protein is obtained. It will be understood by those skilled in the art that the process disclosed herein can be operated with appropriate modifications and variations to obtain the afore-
5 mentioned products. For example, the quinoa grain can be mechanically abraded prior to the step of flaking. The protein fraction obtained after step (4) can be further purified by isoelectric precipitation before step (5), if necessary. The process disclosed herein is designed to maximize isolation of the individual components contained in quinoa grain and thus enables one to obtain other components such as quinoa oil, starch, and fiber
10 at different stages of the process, as illustrated in the flow diagram below.

DETAILED DESCRIPTION OF THE INVENTION

15 In general the terms and phrases used herein have their art-recognized meaning, which can be found by reference to standard texts, journal references and contexts known to those skilled in the art. The following definitions are provided to clarify their specific use in the context of the invention.

20 The term, "quinoa protein concentrate(QPC)", as used herein, is intended to indicate the protein product obtained from quinoa grain having a protein content of at least about 50 wt%, preferably of at least about 70 wt%, most preferably of at least about 90 wt%, on a dry weight basis. The protein content is generally determined by the procedure as described in AACC (2000). Typically, this is determined by kjeldahl
25 nitrogen x 6.25 (N x 6.25) on a dry weight basis.

The term, "functionality", is a well known term in the food industry and relates to physical and chemical properties of food molecules that affect their behavior and produce desired effects in foods during formulation, processing, preparation, and
30 storage (Murano 2003).

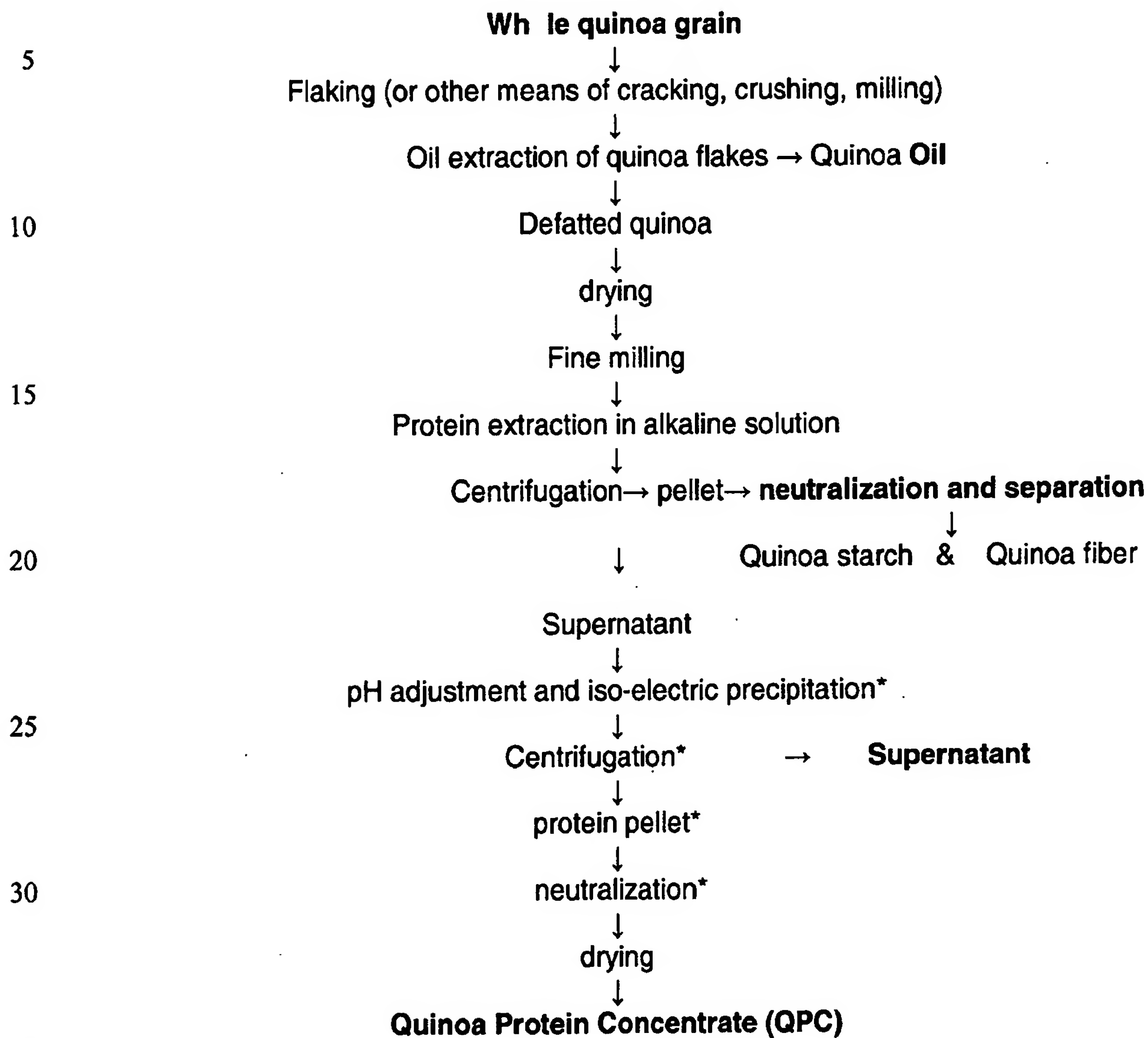
The term, "infant food", more commonly referred to as "food for infants" means any food product intended for use for infants up to one year in age, and generally refers to solid foods for older infants age six months to one year in age. "Foods for toddlers" generally refers to foods for toddlers age one year to two year in age. "Foods for children" refers to foods for pre-school children age 2-5 years and schoolchildren up to 12 years in age. The designation becomes important when estimating amino acid requirements.

Disclosed herein is a new plant protein source termed, quinoa protein concentrate, having a protein content of at least about 50 wt%, on a dry weight basis, and other isolated components contained in quinoa (*Chenopodium quinoa* Chenopodiaceae) grain. Despite the recent interest in quinoa in the food, paper, and cosmetic industries due to its unique starch properties and high lipid content compared to other cereals, quinoa as a plant protein source has not been explored. The inventors herein discovered an efficient process by which maximum amounts of quinoa protein, as well as other isolated components of commercial value such as oil, fiber, and starch contained therein, can be obtained.

The process is illustrated schematically in the following diagram.

20

Fig. 1. Preparation of quinoa protein concentrate from *Chenopodium quinoa*, *Chenopodium* a .



This process provides means to isolate individual components of nutritional and commercial value from quinoa grain. For example, quinoa oil which is present at about 6-9% in unprocessed quinoa seed can be obtained at a level above 90 % from the initial solvent extraction. Likewise, the starch level obtained from the process is 90-100 %. Quinoa fiber isolated from the process is at a level of at least 50%. The steps indicated

with * are optional in isolating quinoa protein concentrate, i.e., one can obtain quinoa protein concentrate of at least about 50 wt% without carrying out the steps indicated.

QPC isolated by the process can be used instead of other plant proteins such as alfalfa proteins, grass proteins, soya proteins and rape proteins, etc., or animal proteins such as milk proteins and meat proteins in pet food and animal feed. QPC can also be used in processed foods, diet foods, health food or nutritional supplements, gluten free products, and as a substitute for wheat and other grains, milk, and eggs. QPC is also useful for nutritional purposes as a source of high quality protein in a wide variety of high-energy food and beverage products (protein bars, protein drinks, nutritional beverages including meal replacement (drinks)).

QPC may be used in conventional applications of protein concentrates, such as, protein fortification of processed foods, emulsification of oils, body formers in baked goods and foaming agents in products which entrap gas. QPC would also be used for a variety of functional effects that are associated with proteins, e.g., as a gelation aid in yogurts and pudding, as a water binder in meat and sausage, as a foaming or whipping aid in toppings and fillings, and as an emulsifier in ice cream, margarine, and mayonnaise. In addition, QPC may be formed into protein fibers, useful in meat analogs, may be used as an egg white substitute or extender in food products where egg white is used as a binder. Other uses of QPC are in edible films and capsules, biodegradable packaging, industrial and cosmetic applications, and in personal care products. QPC can replace all or a portion of the fat or cream in food products such as ice cream, yogurt, salad dressing, mayonnaise, cream, cream cheese, other cheeses, sour cream, sauces, icings, whipped toppings, frozen confections, milk, coffee whitener and spreads. QPC can be hydrolyzed to produce a variety of vegetarian flavors as in the case with hydrolyzed vegetable proteins from soy.

Sample Preparation:

Quinoa grain was harvested and cleaned with sieves and shaking belts to remove stems, rocks, and debris, similar to the manner by which other grains are processed. Optionally, quinoa can further be mechanically abraded, similar to rice polishing, to remove the outer pericarp (or hull) before the next step below.

Fat Extraction:

Whole quinoa grain was flaked, similar to oat flakes, at ambient temperature, using flaker (Series No. 2188 size 18x12 HD, Ross Machine & Mill Supply, Oklahoma City, OK) with a roll gap of .051mm, or similar art-known flaking equipment. Alternatively, whole quinoa grain may be ground, cracked, crushed or milled or a combination thereof, with or without tempering to adjust the moisture content of the grain to achieve the same results. Quinoa flakes were extracted with 1:1 (quinoa:ethanol) using lab Model IV oil extractor, size .25 cu ft (Crown Iron Works, Roseville, MN), or similar art-known oil extraction equipment. Other nonpolar solvents, such as hexane, can be used to extract oil. This step of defatting can be carried out at later steps, for example, after concentrating and drying the protein, if desired. The preferred ratio of quinoa to solvent is about 1:1 (w/v), however, this ratio can be adjusted depending on the solvent and a given sample of flaked quinoa. Quinoa oil micelle and solvent mixture was separated from the quinoa marque (the defatted material containing protein, starch, fiber etc.) using the oil extractor equipment. The solvent was recovered from the quinoa oil and the oil can be refined further according to method of Koziol (1992), similar to corn and soybean oil refining. The quinoa marque was air-dried or dried with mild heat to prevent or minimize damage to protein and starch. Drying removes moisture and residual solvent and what is left is called defatted quinoa.

Protein Extraction:

Ten grams of defatted quinoa was milled finely to about 100 microns or less to yield defatted quinoa flour which is also called oil seed meal. To extract protein, the defatted quinoa flour was suspended in 100 ml of 0.03 mol/l sodium hydroxide (pH of about 10) and stirred mechanically at ambient temperature for about 4 hours to maximize solubility of the protein. The suspension mixture was centrifuged for 30 minutes at 6,000 g at about 0-10 °C. The supernatant ("sup 1") containing protein was separated from the pellet ("pellet 1") containing fiber, starch, and insoluble protein. This separation can be achieved using hydrocyclone separators that are well known in the art.

The optimal ratio of the defatted quinoa flour to alkaline solution is 1:10 (w/v), however, this ratio can be adjusted, if necessary, and the pH can be in the range of 8-11. The temperature is not critical for this step and can be readily modified. The length of the extraction should be adjusted to maximize protein recovery, in our hands, about 4 hours and longer yielded most protein.

The pH of the sup 1 was then adjusted to about 4.25 with hydrochloric acid (any food grade acidulant can be used) in order to precipitate the protein. The pH of the sup 1 can be adjusted in the range of about 6 to 8, preferably about 7.0. Quinoa protein can be prepared from this neutralized protein fraction simply by drying (e.g., concentrating by spray-drying). The quinoa protein thus obtained contains at least 20 wt% protein on a dry weight basis.

Alternatively, quinoa protein can be purified further by isoelectric precipitation using a modified method described by Mohamed *et al.* (2000) Nahrung 44(1):7-12. In this case, the supernatant 1 was adjusted to pH 4.25 using hydrochloric acid to precipitate the protein. The pH for this step can be in the range of 3-6. The pellet containing protein precipitates was separated by centrifugation for 30 minutes at

13, 000 g at about 0-10 °C. The protein pellet can be separated using other means such as hydroclone separators or simply by letting the protein settle over time. The newly obtained pellet can be used as a protein source as it is at this stage. Generally, the protein pellet is resuspended in a small volume of water (e.g., 1 g/10 ml H₂O),
5 neutralized (pH range 4-7) and spray-dried or freeze-dried. The product obtained at this stage contains typically about 90 wt % protein, on a dry weight basis, as determined by micro-Kjeldahl method or Dumas combustion method (AACC 2000). Depending on the exact procedure used to obtain the protein concentrate from quinoa (referred herein as "quinoa protein concentrate"), the protein content ranges from about 50 wt% to at least
10 about 90 wt%.

Starch Extraction:

The pellet 1 obtained as above was resuspended in 100 ml of water. The pH
15 was adjusted to about 5.5 (the range for carbohydrase activity is 3 to 7) and the temperature was increased to about 50°C (the range of carbohydrase activity is 25-70 °C). Carbohydrases (catalyze the breakdown of cell walls, into glucose, cellobiose and higher glucose polymers, Novozymes 2002) were added to the suspension. The pH and the temperature were maintained during the enzyme digestion for about 1 hour.
20 The digest was neutralized and vacuum filtered through a series of wire mesh cloth in order to separate the starch from the partially digested material such as fiber and insoluble proteins. This separation step can be carried out by other equipment cyclones that are known in the starch industry. The digestion step using carbohydrases improves the yield of quinoa starch.

25

Quinoa Oil:

Quinoa has potential to be a greater and more nutritional source of oil than oil produced from cereals crops (Fleming and Galwey 1995). The oil content of quinoa is
30 about 5.6%, with some varieties having lipid contents up to 9.5%. The yield of extractable vegetable oil per hectare could easily exceed that obtained from maize (80-

400 kg/ha and 20-50 kg/ha, quinoa and maize, respectively) making quinoa a valuable new oil crop (Koziol 1990). Quinoa oil is rich in unsaturated fatty acids, although desirable nutritionally, unsaturated fatty acids are unstable to oxidation. However, quinoa oil is quite stable due to high levels of natural antioxidant vitamin E, 690-740 ppm α -tocopherol and 790-930 ppm γ -tocopherol. Although, Koziol found concentrations fall to 450 and 230 ppm, respectively, after refining, 100-200 ppm is sufficient for optimal antioxidant activity of these isomers (Hudson and Ghavami 1984).

High lipid content compared with traditional cereals and essential fatty acid profile make quinoa a potential valuable oil crop. Quinoa oil is a rich source of essential fatty acids linoleic and linolenic, which constitute approximately 55-63% of the oil (Ruales and Nair 1993, Fleming and Galwey 1995), and make it similar to that of soya oil. In a comparison of fatty acids and triacylglycerol compositions, quinoa oil had the lowest saturate/unsaturate ratio compared with oils from five *Amaranthus* accessions, buckwheat, corn, ricebran, sesame, soybean and cottonseed (Jahaniaval and others 2000). In addition, quinoa and soybean oils had the most favorable linoleic to linolenic acid ratio of the preceding oils.

Starch and other carbohydrates:

Studies on the physico-chemical characteristics of quinoa starch have been carried out by Wolf and others (1950), Scarpati de Briceño and Biceño (1982), Atwell and others (1983), Varriano-Marston and de Francisco (1984) and Lorenz (1990). The size of the starch granule and its amylose content are important factors in determining functional properties in food systems. Starch granules occur in the perisperm cells as compound granular aggregates and the individual starch granule, with an average particle size of 1-2 μm (Atwell and others 1983), is small and uniform compared with that of maize and wheat, 1-23 μm and 2-40 μm , respectively (Wolf and others 1950, Swinkels 1985). Although, small starch granules have been shown to have reduced baking potential (Kulp 1973), the small size and uniformity of quinoa starch granules impart a smooth texture and mouthfeel. This attribute has gained considerable interest

from food, paper, and cosmetic industries. Consequently, in 1989 European Patent Application No 89121654.1 was established for the manufacture of a carbohydrate-based cream substitute from quinoa starch. Small quinoa starch granules also have application as inexpensive filler in low density polyethylene films (Ahamed and others
5 1996).

Atwell and others (1983) performed an in-depth characterization of quinoa starch. Analysis indicates 11% amylose content, which is low in comparison to most cereal starches. It is comparable, however, to some varieties of rice, as reported by Williams
10 and others (1958). Lorenz (1990) found that quinoa starch performs poorly in cake and bread baking due to its low amylose content and small starch granule. The researcher also found a higher swelling power of quinoa starch than that of barley, wheat, rice, amaranth, and potato, thus performs well as a thickening agent in fillings.

Free sugars in quinoa were evaluated to contain 4.55%, 2.41%, and 2.39%,
15 glucose, fructose, and sucrose, respectively (González and others 1989). In the same study, the starch level was much lower than that reported by other authors (Ranhotra and others 1993). Consequently, due to high enzyme activity, starch levels will decrease and free sugars will increase upon grinding into flour.

Those skilled in the art will appreciate that the invention described herein is
20 susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred
25 to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

All references cited in the present application are incorporated in their entirety
herein by reference to the extent not inconsistent herewith.

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REPRESENTATIVE CLAIMS

1. A quinoa protein concentrate having a protein content of at least about 50 wt %.
2. A quinoa protein concentrate having a protein content of at least about 70 wt %.
3. A quinoa protein concentrate having a protein content of at least about 90 wt %.
4. A method of processing quinoa (*Chenopodium quinoa*, Chenopodiaceae) grain to isolate oil, fiber, starch, and protein comprising the steps of:
 - (a) flaking the quinoa seeds,
 - (b) extracting the oil from the flakes with a solvent leaving a defatted quinoa marque and separating the oil from the solvent,
 - (c) drying and milling the defatted marque,
 - (d) extracting the protein from the milled marque in alkaline solution,
 - (e) separating solubilized protein from the alkaline solution leaving a pellet containing the fiber and starch,
 - (f) neutralizing the solubilized protein,whereby a quinoa protein concentrate containing at least about 50 wt% protein is obtained.
5. The method of claim 4 further comprising a step of purifying the protein by iso-electric precipitation at an appropriate pH after step (e) but before step (f).
6. The method of claim 5 wherein the pH is in the range of about 4.0 – 8.0.
7. Quinoa starch isolated according to the method of claims 4 or 5.

8. Quinoa fiber isolated according to the method of claims 4 or 5.
9. Quinoa oil isolated according to the method of claims 4 or 5.
10. A product comprising the quinoa protein concentrate of claims 1-3.
11. An animal feed comprising the quinoa protein concentrate of claims 1-3.

ABSTRACT

The present invention relates to a new source of high quality plant protein, termed, "quinoa protein concentrate (QPC)", which contains at least about 50 wt % protein and methods of preparing such protein concentrates as well as starch, oil, and fiber from quinoa grain. The quinoa protein concentrate of the invention is useful as food additives, pet food additives, and animal feed supplements.

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Applicant Information

Applicant Authority type:: Inventor
Primary Citizenship Country:: US
Status:: Full Capacity
Given Name:: Laurie
Family Name:: SCANLIN
City of Residence:: Arvada
State or Province of Residence:: CO
Country of Residence:: US
Street of mailing address:: 13842 W. 68th Way
City of mailing address:: Arvada
State or Province of mailing address:: CO
Country of mailing address:: US
Postal or Zip Code of mailing address:: 80004

Applicant Information

Applicant Authority type:: **Inventor**
Primary Citizenship Country:: **US**
Status:: **Full Capacity**
Given Name:: **Martha**
Family Name:: **STONE**
City of Residence:: **Fort Collins**
State or Province of Residence:: **CO**
Country of Residence:: **US**
Street of mailing address:: **1313 Centennial Road**
City of mailing address:: **Fort Collins**
State or Province of mailing address:: **CO**
Country of mailing address:: **US**
Postal or Zip Code of mailing address:: **80525**

Correspondence Information

Correspondence Customer Number:: **23713**
Name:: **Greenlee, Winner and Sullivan, P.C.**
Street of mailing Address:: **5370 Manhattan Circle, Suite 201**
City of mailing address:: **Boulder**
State or Province of mailing address:: **CO**
Country of mailing address:: **US**
Postal or Zip Code of mailing address:: **80303**
Phone number:: **303-499-8080**
Fax number:: **303-499-8089**
E-Mail address:: **winner@greenwin.com**

Representative Information

Representative Customer Number:	23713
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